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Structure and Activity in a Series of 2-Halogenoalkylamines

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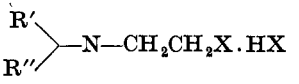
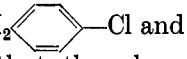
In a classical paper, Nickerson and Gump[1] discussed the relation between structure and activity in compounds related to Dibenamine. They reported a difference in potency between the *p*-chloro and *m*-chloro forms of *N*-ethyl-*N*-2′-chloroethyl-chlorobenzylamine. An attempt has been made to explore the relation between the position of the halogen substituent on the benzyl ring for chloro-, bromo-, and iodo-ethylamines bearing a *p*-, *m*- or *o*- chlorobenzyl group on the nitrogen atom, and to explain the findings in the light of current theory.[2] The basic structure of these compounds is



where R′ = ethyl, R″ =  and X = halogen.

It is generally agreed that the pharmacological activity of 2-halogenoalkylamines follows upon the formation of ethyleneiminium ions[3] which are the active chemical species. Certain of these have been isolated[4] and their properties examined. Certain other compounds of the above general structure in which R′ = R″ = phenoxyethyl-, *o*-tolyloxyethyl-, and *p*-tolyloxyethyl- have been examined in order to explore relations in this series further.[4] A considerable degree of antiadrenaline activity having been attributed to the structure where R′ = ethyl, R″ = 9-fluorenamine and X = Cl[5–12], a full series of related compounds is included. Additionally, certain compounds where R′ = benzyl, R″ = thienylamine or R′ = ethyl, R″ = thionaphthenylmethylamine were examined because of reported activity[13,14] in the

chloro- compound. The latter molecules form in solution a considerable proportion of dimer; this property was utilized to test the hypothesis that dimer rather than ion is the active form of halogenoalkylamines.

In addition to antagonism to selected actions of adrenaline, noradrenaline, 5-OH tryptamine, histamine and acetylcholine, the toxicity, local anaesthetic potency and the effects on the behaviour and E.E.G. pattern, heat control and other central activities of small animals were investigated in order to examine the relation between antiadrenaline activity and the other properties, and in particular to determine if there was any evidence of central depressant action. A detailed study of the antihistamine action of one compound (AT_{11}) was made in order to compare its mode of action with that of mepyramine, which is currently a controversial topic,[15] and to examine the usefulness of the methods employed. The action of selected compounds on the potential difference across isolated frog skin was examined in order to detect the type of adrenaline receptor in that tissue.

Methods

Chemical

In all cases the compounds were pure crystalline salts, stable as such in the dark but not in solution. Appropriate amounts were dissolved in 0·05–0·1 ml acetone and diluted with 0·9 per cent (w/v) saline. Solutions were kept on ice and used within 15 min. No alkali was added. Phosphate buffer–saline 50 : 50 (v/v) was used as solvent where a pH of 7·2 was desirable. For the kinetic-pharmacology experiments a technique described previously was applied.[16]

Pharmacological

Toxicity was measured by Karber's method[17] in groups of ten white mice injected intraperitoneally with buffered solutions. When solubility proved to be low, propylene glycol–acetone 50 : 50 (v/v) was used as solvent in minimal quantity and saline added. The toxicity of this solution was checked. A suspension with

tragacanth was also used for a few compounds. *Behavioural studies* were made in groups of ten mice injected with 0·1 ml volumes of buffered solutions of selected compounds[18] into the cerebral ventricle and in mice, rats, guinea pigs and rabbits injected intravenously. Selected compounds were tested for antagonism to amphetamine.[19] The effect of 5 mg/kg of AT_2 injected intravenously on the sleeping time of groups of ten mice kept at a temperature of 30° and given 65 mg/kg pentobarbitone sodium intraperitoneally, or 15 mg/kg of reserpine was determined; the effect on the LD_{50} of leptazole was also determined.[17] Analgesia was tested for in groups of young rats;[20] morphine was used as a standard. Twenty-four male rats of 100 g weight were injected intravenously, half with saline, half with 25 mg/kg AT_2 and kept individually for 6 h at 4° after which the rectal temperature of each was recorded to $\pm 0 \cdot 1°$.

Three pin electrodes were driven to a depth of 0·5 cm through the outer table of the skull in six rabbits anaesthetized with 30 mg/kg pentobarbitone sodium and in four anaesthetized with 20 mg/kg thiopentone sodium and the animals allowed to recover. The midpin was placed in the midline sagittal plane between the eyes and the other two 1 cm anterior and posterior. Electroencephalograms were recorded as in Fig. 1. Alternatively the front electrode was midline 0·5 cm anterior to the position of the middle electrode of pattern 1 and the other two posteriorly in coronal section at a distance of 1 cm. The positions and depths of the electrodes were subsequently checked by X-rays in two planes and by dissection and an approximate location for the tips of the electrodes made as follows.

Pattern 1. Electrode (a) nasal passage, (b) frontal lobe, (c) temporal lobe.

Pattern 2. Electrode (a) nasal passage, (b) frontal lobe, (c) frontal lobe.

The E.E.G. was recorded before and at various intervals after intravenous injection of 5 mg/kg of compound AT_2.

Antagonism to adrenaline (1 μg/kg) and *noradrenaline* (0·5 μg/kg) was measured on the blood pressure of atropinized spinal rats injected with 5 mg/kg of hexamethonium bromide.[3] The ED_{50} was determined from a plot of the dose-response, the effect of the doses being taken as additive. The period of time necessary to establish fully the action of the individual compound (5–15 min)

was determined. Five rats were used to determine the ED_{50} of each compound from a plot of four doses, which were taken to be additive in effect, and to determine each point on the curve relating antiadrenaline activity to age of the solutions of AT_{11} used for kinetic-pharmacology studies. The ED_{50} of each compound for 5-hydroxytryptamine was also determined on five rats; three dogs each were used to determine the dose of selected compounds needed to abolish the pressor response to adrenaline (4 μg/kg) and to noradrenaline (2 μg/kg).[3] The effect of single injections of selected compounds into the perfused vessels of the isolated rabbit ear on the constrictor activity of adrenaline (1 μg) and noradrenaline (0·5 μg) was recorded.[21]

The potential difference maintained across isolated abdominal skin of *Rana temporaria*[22] mounted at 22° in Frog-Ringer solution of pH 7·8 containing bicarbonate was measured with a high impedance potentiometer (Vibron Electrometer). Drugs dissolved in Frog-Ringer solution were added to the inside of the skin as required, 1 h after the preparation was set up. The effects of adrenaline, noradrenaline and isoprenaline at a concentration of 10^{-6} base on skin potential and of compound AT_2 on their actions were determined.

Antagonism to histamine was measured by the guinea pig aerosol method.[16] An ED_{50} for each compound acting for 30 min was obtained in groups of ten animals. A selected compound (AT_{11}) was further investigated in detail on guinea pig ileum in Tyrode's solution at 37° in a semi-automatic, 10-ml isolated organ bath. Varying concentrations of AT_{11} or of mepyramine were added and the responses to a standard dose of histamine noted over a number of hours during which recovery was studied. Increasing amounts of the compounds were each allowed to act for time t and the dose of histamine needed to restore the initial response determined. The dose ratio[23] was measured at two values of t and over a range of values of antagonist concentration, the value of the agonist concentration being kept constant. Five animals were used for each determination; the two drugs AT_{11} and mepyramine were used, and the two values of t obtained on adjacent segments of one ileum. Further studies were made to determine possible interaction between mepyramine, AT_{11}, histamine, K^+, adrenaline, acetylcholine and 5-OH tryptamine.

Local anaesthetic properties were investigated by the wheal method in guinea pig skin[24] or by application of solutions to the conjunctival sac of the rabbit.[18]

Cholinesterase. The effect of several concentrations of compounds AT_2 and AT_{11} (buffered), of mepyramine maleate and of the solvents and media used on cholinesterase activity in homogenate of whole rat brain was determined by the conventional gas-manometric method, using ACh as substrate.

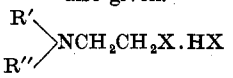
Neuromuscular junction. The action of compound AT_2 was investigated on five preparations: (1) the isolated frog rectus stimulated with ACh or KCl; (2) the isolated frog gastrocnemius in Ringer's solution stimulated directly with square pulses at 12/min 5V 1 msec; (3) the isolated rat diaphragm-phrenic nerve (stimulated indirectly at 6 or 12 pulses/min 10V 1 msec; (4) the gastrocnemius-sciatic nerve preparation in urethanized rats injected via the aorta and stimulated as in (5); and (5) the tibialis anterior of the chloralosed cat indirectly stimulated at 10 pulses/min 5V 1 msec and injected close arterially. Comparisons were made with the action of K^+, suxamethonium HBr and gallamine triethiodide and the effects of neostigmine were examined.

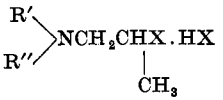
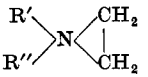
Results

Toxicity

The structures and molecular weights of the compounds examined are shown in Table I and the LD_{50} at 1 h for selected compounds. These compounds have a double action. All are depressants of the central nervous system. This is shown by the animals becoming still, with slow respiration and weakness of the hind-legs. Superimposed on this pattern of response is a phase of jactitation, especially with compound JFA_{29}. As asphyxia develops, death is quiet and delayed deaths at 24 h occur with JFA_8, JFA_9, JFA_{10}, P_1, JFA_{35} and AT_2. JFA_3 is the quickest in onset of symptoms which are purely depressant. AT_8 is notable for the degree of muscular weakness produced. P_1, the iminium cation of JFA_{8-10}, resembles the parent compounds. Sodium picryl sulphonate is not toxic in a dose of 0·25 g/kg i.p. which is greater than the amount in the lethal doses of P_1.

Table I. The code numbers, structures and molecular weights of the compounds under investigation. The LD_{50} in male white mice for selected compounds is also given.[17]



Compound code no.	R′	R″	X	Mol. wt.	LD_{50}
JFA_8	*p*-chlorobenzyl	Et	Cl	268	350
JFA_9	*p*-chlorobenzyl	Et	Br	402	80
JFA_{10}	*p*-chlorobenzyl	Et	I	543	130
JFA_{11}	*m*-chlorobenzyl	Et	Cl	268	
JFA_{12}	*m*-chlorobenzyl	Et	Br	402	
JFA_{13}	*m*-chlorobenzyl	Et	I	543	
JFA_{14}	*o*-chlorobenzyl	Et	Cl	268	
JFA_{15}	*o*-chlorobenzyl	Et	Br	402	
JFA_{16}	*o*-chlorobenzyl	Et	I	543	
JFA_{28}	2′-phenoxyethyl	as R′	Cl	356	
JFA_{29}	2′-phenoxyethyl	as R′	Br	445	90
JFA_{30}	2′-phenoxyethyl	as R′	I	539	
JFA_{31}	2′-*o*-tolyloxyethyl	as R′	Cl	370	
JFA_{32}	2′-*o*-tolyloxyethyl	as R′	Br	459	250
JFA_{33}	2′-*o*-tolyloxyethyl	as R′	I	553	
JFA_{34}	2′-*p*-tolyloxyethyl	as R′	Cl	370	
JFA_{35}	2′-*p*-tolyloxyethyl	as R′	Br	459	600
JFA_{36}	2′-*p*-tolyloxyethyl	as R′	I	553	
AT_1	9-fluorenyl	Et	Cl	308	
AT_2	9-fluorenyl	Et	Br	397	190
AT_3	9-fluorenyl	Et	I	491	
AT_4	9-fluorenyl	benzyl	Cl	370	
AT_5	9-fluorenyl	benzyl	Br	459	500
AT_{10}	3-thionaphthenyl-methyl	Et	Cl	290	
AT_{11}	3-thionaphthenyl-methyl	Et	Br	379	130
AT_{12}	3-thionaphthenyl-methyl	Et	I	473	
AT_{13}	2-thienyl	benzyl	Cl	302	
AT_{14}	2-thienyl	benzyl	Br	391	600
AT_{15}	2-thienyl	benzyl	I	485	
					
AT_7	9-fluorenyl	Et	Cl	322	
AT_8	9-fluorenyl	Et	Br	411	190
					
$P_1(JFA_8)$	*p*-chlorobenzyl	Et	picryl sulphonate	489	75
$P_2(JFA_{11})$	*m*-chlorobenzyl	Et		489	
$P_3(JFA_{14})$	*o*-chlorobenzyl	Et		489	

Central Nervous System

An attempt was made to relate biological antagonism of histamine, acetylcholine, 5-OH tryptamine, adrenaline and noradrenaline to central depressant action. Two naphthylmethyl derivatives (J_{11} and J_{13}), the properties of which have been reported previously,[16] were selected because J_{11} is an active antiadrenaline and antihistamine and J_{13} is entirely inactive; the 9-fluorenyl compound (AT_2) was selected because of its great antiadrenaline potency and thionaphthylmethyl compound (AT_{11}) because of its great antihistamine potency. In these compounds the other substituent on the nitrogen atom is ethyl and the halogen bromine, with the deliberate exception of J_{13} where it is fluorine. This element renders J_{13} unreactive and inactive. Differences in molecular weight are allowed for. All compounds are poorly soluble in water. J_{13} is a convulsant by all tests. All are inactive against acetylcholine on isolated guinea pig ileum. Many potent antihistamines are central depressants but antagonism to histamine does not correlate with sedative powers in these compounds, nor does antagonism to the pressor action of 5-hydroxytryptamine. In so far as antagonism to adrenaline–noradrenaline is concerned, AT_2 is some five times stronger than J_{11} and twenty times stronger than AT_{11}. When buffered solutions of the compounds are injected intravenously in small animals and a score made of the effect on mobility, vociferousness, alertness, wildness, tremor, jactitation and other aspects of behaviour, AT_2 is the only consistent sedative. Intracerebral injection in mice mostly produces effects of depression. AT_2 is the most effective antagonist to the lethal action of amphetamine sulphate and this test[19] distinguishes clearly between AT_2 and J_{11}. Injected intravenously in a dose of 5 mg/kg, AT_2 is synergistic with pentobarbitone sodium. In mice given reserpine, 15 mg/kg intraperitoneally, there is no apparent difference in the degree or duration of action of AT_2 given intravenously. None of these compounds is an analgesic. AT_2 decreases and J_{13} increases the lethality of leptazole. AT_2 in doses of 25 mg/kg causes a fall in body temperature in young rats kept in the cold.

The E.E.G. pattern of normal rabbits prepared as described is invariably modified by intravenous injection of AT_2. In recordings from electrode pattern 1 (midline sagittal), there was a decline in voltage but no change in rate in channel 1, whereas in channel 2

there was a decline from 84 to 33 spikes/min and from +200 μV to +150 μV. Barbiturate anaesthesia exaggerated this. With electrode pattern 2 (anterior midpoint to temporal lobes coronal) there was a decline in voltage but no change in rate in positive

Table II. Relationships of selected 2-halogenoalkylamines to drugs and their effect on behaviour. Antagonism to ACh on guinea pig ileum is recorded as an ED_{50} in mg/l.; to adrenaline and noradrenaline as an ED_{50} in mg/kg,[3] as also for 5-hydroxytryptamine; antagonism to histamine as an ED_{50} subcutaneously against histamine aerosol in guinea pigs;[16] to amphetamine sulphate as an ED_{50} in mg/kg;[19] relation to leptazole as a percentage difference in LD_{50};[17] to hypnotics as a percentage change of sleeping time in groups of 10 male white mice injected with pentobarbitone sodium 65 mg/kg or reserpine 15 mg/kg 30 min previously; analgesia on the tail reflex,[20] and finally an observational assessment of the effect on behaviour in mice after intravenous and intracerebral injection and on rats, guinea pigs and rabbits injected intravenously. The most strongly sedative is the most potent antagonist of adrenaline–noradrenaline

	AT_2	AT_{11}	J_{11}	J_{13}
Mol. wt.	397	379	292	231
Solubility in water	Very poor	Poor	Poor	Poor
Structure R″	9-fluorenyl	Thionaphth-enylmethyl	Naphthyl-methyl	Naphthyl-methyl
Adrenaline	0·03	0·60	0·16	25
Noradrenaline	0·04	0·97	0·21	25
5-OH trypt-amine	1·60	0·37	0·28	25
Histamine	5·00	0·03	0·03	25
Acetyl-choline	5	5	5	5
Amphetamine	2·40	5·40	Inactive	Convulsant
Effect on behaviour i.v.	Strong sedative	Weak sedative	Weak sedative	Convulsant
i.c.	±effects	Stimulant	Stimulant	Convulsant
LD_{50} i.p.	70	40	45	300
Pento-barbitone	+40%	+5%	+7%	−18%
Reserpine	No effect	No effect	No effect	No effect
Leptazole	−10%	No effect	No effect	+20%
Analgesia	No effect	No effect	No effect	No effect

potential spikes in channel 1 and a loss of slow negative waves, while in channel 2 voltage declined but rate increased. Anaesthesia again exaggerated the depressant changes (see Fig. 1).

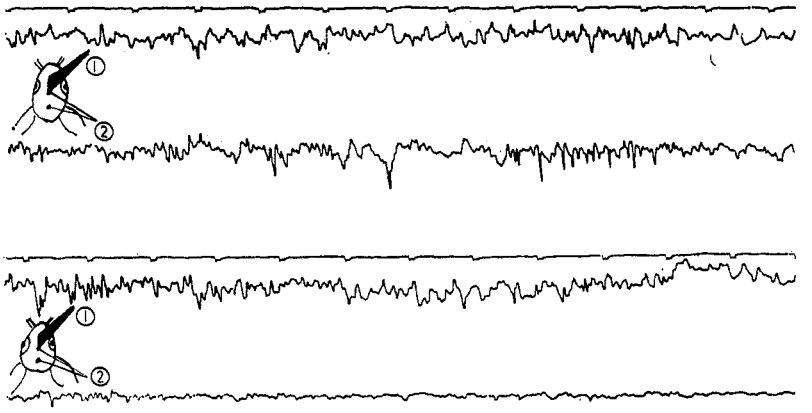


Fig. 1. Rabbit, male 3·2 kg, pentobarbitone sodium 30 mg/kg E.E.G. recorded on 2 channels from 3 pin electrodes inserted 0·5 cm into nasal passage, frontal and temporal lobes. Upper record is control sleep pattern, lower record is depression in cortical activity 5 min after i.v. injection of 5 mg/kg of compound AT_2. Time interval is in sec.

Antagonism to Adrenaline, Noradrenaline and 5-Hydroxytryptamine

The figures in mg/kg and μM/kg representing the ED_{50} of the compounds against the pressor effects of adrenaline and noradrenaline are given in Table III with phenoxybenzamine and the naphthylmethylhalogenoalkylamine J_{11} for comparison. All the compounds examined antagonize the pressor action of adrenaline in smaller doses than are needed to antagonize equipressor noradrenaline. The ratio of the ED_{50} NA/A is always greater for a given compound tested in an intact anaesthetized animal (cat, dog or rat) than it is in a spinal animal pretreated with ganglion blocking agents; e.g. from Table III for compound JFA_{11}, it is 4·0 in the dog and 1·03 in the spinal rat. This is a measure of the difference in vascular tone of the two preparations. The pressor component of the response to the two amines is equally effectively antagonized by the 2-halogenoalkylamine.

The antagonistic activities of the compounds to the pressor action of 5-hydroxytryptamine vary and JFA_{13}, for instance, is only weakly active; against the activities of the two pressor amines, the compounds are equi-active. The rate of onset and duration

Table III. Antagonism to the pressor effect of adrenaline (1 μg/kg) and noradrenaline (0·5 μg/kg) in the atropinized spinal rat pretreated with C_6 5 mg/kg. i.v., expressed as an extrapolated ED_{50} in mg/kg (and μM/kg in parenthesis). For important compounds the ED_{50} against 4 μg/kg adrenaline and equipressor noradrenaline in the atropinized dog anaesthetized with pentobarbitone sodium 30 mg/kg is also stated. The antagonism to the pressor effect of 20 μg/kg of 5-hydroxytryptamine on different rats similarly prepared is stated for selected compounds

Compound code no.	Spinal rat, ED_{50}			Anaesthetized dog, ED_{50}	
	Adrenaline	Noradrenaline	5-OH tryptamine	Adrenaline	Noradrenaline
JFA_8	9·80 (36·6)	10·60 (39·6)	26·0 (97·0)	3·0	6·5
JFA_9	1·41 (3·5)	2·15 (5·34)	4·2 (10·4)		
JFA_{10}	2·25 (4·14)	2·64 (4·86)	3·23 (5·94)		
JFA_{11}	1·31 (4·88)	1·35 (5·03)	13·0 (48·5)	1·2	5·0
JFA_{12}	0·86 (2·13)	0·89 (2·21)	2·72 (6·76)		
JFA_{13}	0·89 (1·63)	0·77 (1·41)	50 (92)		
JFA_{14}	6·43 (23·9)	5·93 (22·1)	11·70 (43·6)	2·0	4·0
JFA_{15}	0·83 (2·06)	1·04 (2·58)	2·02 (5·02)		
JFA_{16}	0·80 (1·47)	1·13 (2·08)			
JFA_{28}	1·63 (4·57)	2·70 (7·58)			
JFA_{29}	0·10 (2·24)	0·20 (4·49)	0·14 (3·14)		
JFA_{30}	0·16 (2·96)	0·30 (5·56)			
JFA_{31}	1·10 (2·97)	1·33 (3·59)			
JFA_{32}	0·46 (1·0)	0·68 (1·48)	0·143 (0·31)		
JFA_{33}	2·17 (3·91)	3·77 (6·81)			
JFA_{34}	2·70 (7·29)	4·60 (12·43)			
JFA_{35}	0·80 (1·74)	1·27 (2·76)	0·97 (2·11)		
JFA_{36}	0·66 (1·19)	1·10 (1·98)			
AT_1	0·10 (0·32)	0·14 (0·45)	4·20 (13·6)	0·15	0·7
AT_2	0·026 (0·06)	0·04 (0·10)	1·40 (3·52)	0·03	0·1
AT_3	0·013 (0·026)	0·02 (0·04)	1·80 (3·66)		
AT_4	2·75 (7·43)	2·76 (7·45)			
AT_5	0·87 (1·89)	1·10 (2·39)	2·80 (6·1)		
AT_{10}	0·40 (1·38)	0·56 (1·93)			
AT_{11}	0·59 (1·55)	0·97 (2·55)	0·37 (0·97)		
AT_{12}	0·63 (1·33)	1·14 (2·41)			
AT_{13}	9·00 (29·8)	10·0 (33·1)			
AT_{14}	0·95 (2·42)	7·70 (19·7)	0·37 (0·94)		
AT_{15}	0·57 (1·17)	1·05 (2·16)			
AT_7	0·84 (2·6)	1·40 (4·34)			
AT_8	0·027 (0·06)	0·028 (0·06)	0·78 (1·89)		
$P_1(JFA_8)$	0·60 (1·22)	0·84 (1·71)	1·10 (2·84)		
$P_2(JFA_{11})$	0·42 (0·85)	0·57 (1·16)	0·984 (2·01)		
$P_3(JFA_{14})$	0·32 (0·66)	0·48 (0·98)	0·78 (1·59)		
J_{11}(SY28)	0·22 (0·75)	0·29 (0·98)	0·38 (1·29)	0·10	0·2
Phenoxybenzamine	0·137 (0·40)	0·168 (0·49)	0·49 (1·44)	0·10	0·15

of antagonism to adrenaline–noradrenaline and to 5-hydroxytryptamine are about the same and a long-lasting antagonism to the latter drug can be obtained readily if a suitable compound is selected. Some of the 2-halogenoalkylamines exert a powerful antagonism to adrenaline and a relatively weak one to 5-hydroxytryptamine, e.g. AT_3 or *N*-2′-iodoethyl-*N*-ethyl-9-fluorenamine where the ratio of the ED_{50} 5HT/A is 140, whereas in others the relative activities are reversed, e.g. AT_{14} or *N*-benzyl-*N*-2′-bromoethyl-2-thenylamine where the ratio 5HT/A is 0·39. Generally, specificity is low.

The known peripheral site of action of the compounds was confirmed by antagonism of the vasoconstrictor action of adrenaline and noradrenaline on the perfused vessels of the rabbit ear. Compound AT_2 was the most active of those selected and was almost equally active against either amine. A single injection of 100 μg into the perfusing tube can produce an absolute block of the vascular muscle for over 4 h. The affinity of this compound for the tissue must be considerable.

Frog Skin

The inside of the isolated skin of the frog was always electrically more positive than the outside. The value of this potential difference (P.D.), low at first, rose steadily and became stable after 30 min–1 h at a level which varied widely (10–120 mV) but was constant for the individual skin. Compound AT_2 (*N*-2′-bromoethyl-*N*-ethyl-9-fluorenamine) dissolved in a minimal volume of acetone–water and diluted with Frog-Ringer (pH 7·8) in a final concentration of 10^{-5} caused a fall in skin P.D. of some 15 per cent without change in pH. Repeated washing for 20 min did not restore the initial P.D. Adrenaline and isoprenaline caused a fall in P.D. (over 20 per cent) after 15 min. This represents the stable situation and ignores initial fluctuations. Noradrenaline caused a rise in P.D. which was more marked initially (plus 33 per cent) than at 15 min (plus 14 per cent) and in some cases the P.D. fell below the initial level. The fluctuation in P.D. across isolated frog skin which follows addition of adrenaline has been shown[22] to be due to stimulation of two mechanisms—Na^+ and Cl^- transport. The difference in response

to these amines is probably a question of the relative preponderance of stimulation of these. The initial value of P.D. reflects their relative importance in the individual skin and this determines the final state. The higher the initial P.D. the greater is the fall after the addition of amine.

Compound AT_2 inhibits the stimulant action of the amines on these two transport mechanisms in frog skin with equal facility, if given before the amine and in adequate amount. Prior administration of the amine inhibits the development of blockade of the tissue. These points are illustrated in Table VI and these relations are characteristic of 2-halogenoalkylamines and adrenaline. The partial nature of the change in P.D. was confirmed terminally by addition of cyanide or by gassing the solution with 5 per cent carbon dioxide, both of which procedures bring about a profound fall in P.D.

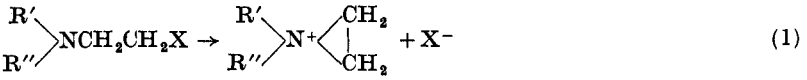
Table IV. The potential difference in mV maintained across isolated abdominal skin of *Rana temporaria* in bicarbonate Frog-Ringer and the effect at 15 min as a percentage of the initial value, of adding adrenaline, noradrenaline or isoprenaline (10^{-6}) to the inside. The 2-halogenoalkylamine compound AT_2 abolishes the effect of the amines on the Na^+ transport mechanism on which this P.D. depends,[22] if the halogenoalkylamine is added before, but not after, the pressor amine

P.D.	Amine	Effect	AT_2 (after amine)	Repeat amine
52	Adrenaline	−27	−12	−33
36	Noradrenaline	+14	− 6	−10
34	Isoprenaline	−21	− 7 (before amine)	−16
65	Adrenaline	− 3	−12	-
50	Noradrenaline	0	− 4	-
33	Isoprenaline	0	−16	-

The Active Form

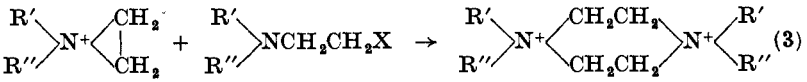
It has been shown previously[3] that a relation can be demonstrated between the antiadrenaline activity of some of these compounds and the amount of E^+ (iminium ion) present in the solution of the compound, which indicates that the active chemical

species for antiadrenaline activity in the 2-halogenoalkylamines is the iminium ion (1).



(1)

(2)



(3)

It is known that addition of water results in formation of an amino alcohol (2) and complete loss of activity. The suggestion has been made that the active form might be the piperazinium form (3). The alcohols of some 2-halogenoalkylamines have been synthesized and shown to be inactive but there has been difficulty in obtaining

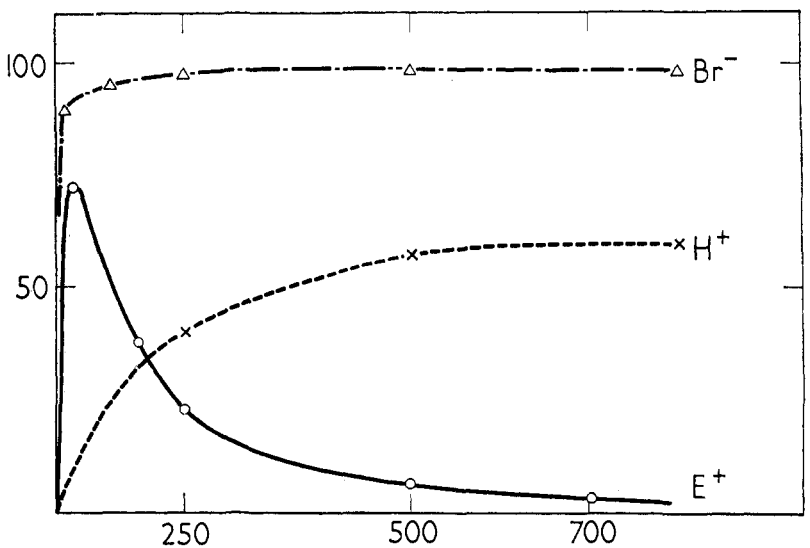


Fig. 2: The decomposition of *N*-2′-bromoethyl-*N*-ethyl-2- thionaphthenylmethylamine (AT_{11}) dissolved in acetone–water, just neutralized, kept at 30° and aliquots analysed at intervals.[4] Antiadrenaline activity of extracts of such a solution is initially high, falls with declining content of iminium ion and is absent at 18 h when ion is absent but alcohol and piperazinium are present

pure piperazinium salts. Another approach is to utilize an aged neutral solution in water of a compound such as AT_{11} (*N*-2′-bromoethyl-*N*-ethyl-2-thionaphthenylmethylamine). As may be seen from Fig. 2, this compound is readily soluble in acetone–water (much less readily soluble in water) and forms 72 per cent of the possible E^+ within 15 min with appropriate liberation of Br^-, which eventually proceeds to almost 100 per cent. The compound therefore mainly undergoes reaction (1) above. The amount of free E^+ falls off and the free H^+ rises as reaction (2) proceeds, but after 18 h, when free ion is absent, there is a significant discrepancy in free H^+. This is almost certainly accounted for by reaction (3).[4] A suitably aged solution of compound AT_{11} (18 h at 30°) may be extracted twice with ether to remove any unreacted parent compound and tested for antiadrenaline potency. There is no activity at 18 h and about half the initial potency at 3 h. The piperazinium ion cannot therefore be active against adrenaline.

Antagonism to Histamine

The relative potencies of selected compounds are shown in Table V. Mepyramine maleate is included as a standard. All

Table V. The antihistamine potencies of selected 2-halogenoalkylamines designated by their code numbers (see Table I for relevant structures) measured as ED_{50} injected subcutaneously in groups of guinea pigs 30 min before 0·5 per cent histamine base as aerosol.[3] The relative potencies of the same compounds as local anaesthetics tested intradermally in guinea pigs[24]

Code no.	ED_{50} mg/kg histamine aerosol	Local anaesthesia intradermal
JFA_8	2·0	0·75
JFA_9	0·5	0·42
JFA_{10}	0·2	0·55
JFA_{29}	3·6	1·10
JFA_{32}	8·0	0·48
JFA_{35}	7·0	0·90
AT_2	5·0	0·80
AT_5	7·4	0·32
AT_8	6·5	0·84
AT_{11}	0·03	0·81
AT_{14}	2·2	0·37
Mepyramine	0·04	–
Procaine HCl	–	1·0

the compounds tested were active, AT_{11} or *N*-2′-bromoethyl-*N*-ethyl-3-thionaphthenyl-methylamine HBr being of the same order of potency as mepyramine (ED_{50} of AT_{11} $8 \cdot 6 - 10^{-5}$ mM/kg and ED_{50} of mepyramine maleate 10^{-4} mM/kg). The most potent antagonist of histamine (AT_{11}) is not the most potent antagonist of adrenaline (AT_2).

The nature of the antagonism to histamine exerted by 2-halogenoalkylamines has been the subject of intensive study.[15]

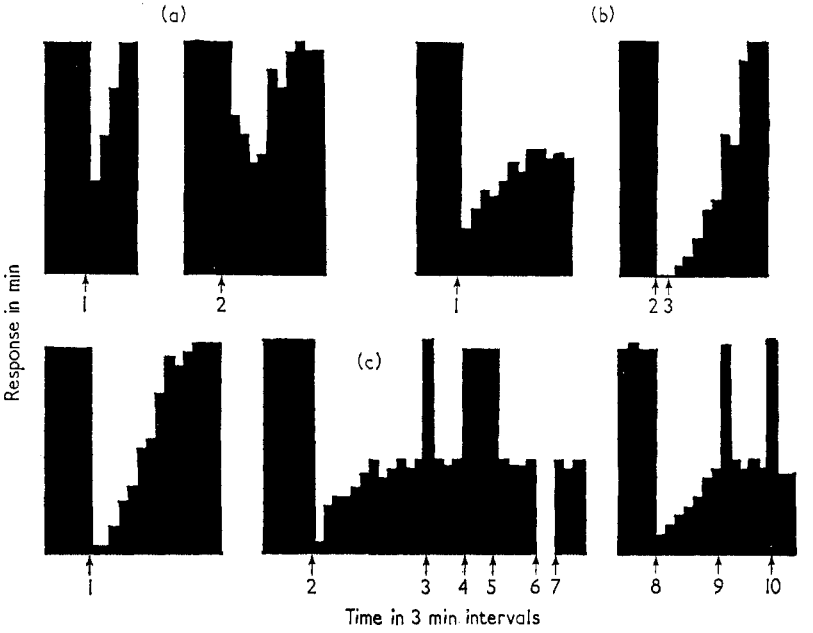


Fig. 3. Antagonism of mepyramine maleate, *N*-2′-bromoethyl-*N*-ethyl-3-thionaphthenylmethylamine HBr (AT_{11}) and *N*-2′-ethyl-*N*-2-naphthylmethyl bromoethylamine HBr (J_{11}) to submaximal doses of histamine on the isolated guinea pig ileum. (a) 1, mepyramine 5×10^{-11}M; (a) 2, equimolar AT_{11}, both for 15 sec; histamine 10^{-8} every 3 min with wash after 30 sec. The antihistamine action is of rapid onset and is reversible at this dose level. (b) 1, AT_{11} 10^{-10}M for 2 min, histamine 10^{-8} every 3 min; (b) 3, as in (b) 1 but after mepyramine 10^{-8}M for 1 min at (b) 2. Mepyramine prevents the long duration of action of AT_{11}. (c) 1, mepyramine 10^{-8}M for 3 min, rapid and complete recovery; (c) 2, AT_{11} 10^{-9}M for 3 min, partial recovery only. (c) 3, histamine dose × 8; (c) 4, $K^+ \times 2$; (c) 5, K^+ normal; (c) 6–(c) 7, an interval of 426 min with regular washing; (c) 8, J_{11} 10^{-9}M; (c) 9, histamine dose × 2; (c) 10, $K^+ \times 2$. Increased histamine or K^+ restores the initial response

Accordingly the properties of AT_{11}, the most active member of the series, have been further investigated.

(*a*) AT_{11} and mepyramine antagonize the same effects of histamine to a similar degree.

(*b*) The onset of action is rapid with both drugs (see Fig. 3 (a)).

(*c*) The effect of mepyramine is reversible over a range of effective concentrations (5×10^{-11}M to 5×10^{-7}M); the effect of AT_{11} may increase on washing and is only partially reversible (see Fig. 4 (a)). Increasing the dose increases the irreversibility.

(*d*) When the time of exposure to the antagonist is increased the

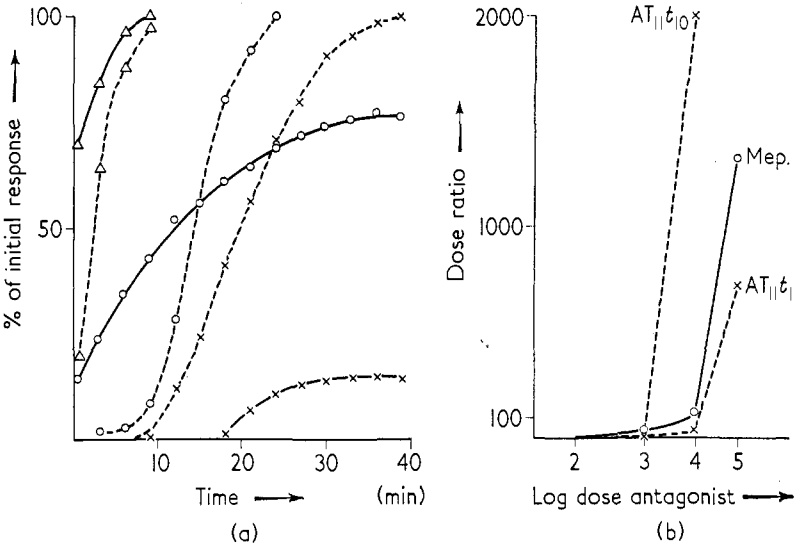


Fig. 4. Antagonism of mepyramine maleate and *N*-2-bromoethyl-*N*-ethyl-3-thionaphthenylmethylamine HBr (AT_{11}) to histamine on the isolated guinea pig ileum. *Section* (*a*). The effect of varying doses of antagonist for a fixed time on the rate and degree of recovery from a standard exposure to histamine. Broken lines mepyramine, unbroken lines AT_{11}. △—△ 5×10^{-11}M, ○--○ 5×10^{-10}M, X--X 5×10^{-9}M. *Section* (*b*). Log dose antagonist (mepyramine and AT_{11}) plotted against dose-ratios for two durations of exposure ($t_1 = 1$ min, $t_{10} = 10$ min), agonist being constant histamine 10^{-8}. Broken line is AT_{11}, the potency of which increases over ten-fold with increase in time of exposure to tissue; unbroken line is mepyramine, the potency of which is maximal by t_1

Neuromuscular Junction

Compound AT_2 exerts a depressant action on muscle as may be seen in the isolated frog gastrocnemius preparation stimulated directly (Fig. 5A). This is not a specific effect of the ethyleneiminium ion as it is also caused by incubated alkaline solutions of

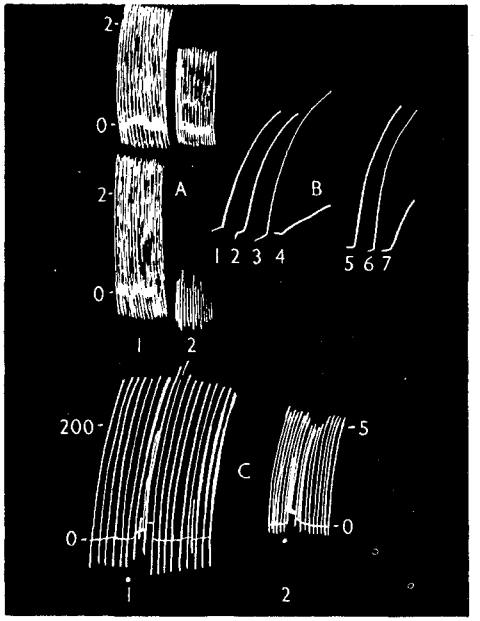


Fig. 5. Traced records illustrating action of *N*-2′-bromoethyl-*N*-ethyl 9-fluorenamine HBr (AT_2) on muscle. *Section A.* Directly stimulated isolated frog gastrocnemius. Between 1 and 2, interval of 20 min and AT_2 10^{-5} to lower muscle of pair. *Section B.* Isolated frog rectus. 1-4ACh 10^{-7}, between B2–B3, AT_2 5×10^{-8}; B3–B4, AT_2 2×10^{-5}; B5–B7, KCl 10^{-3}, between B6–B7, AT_2 2×10^{-5}. *Section C.* Cat, 2·3 kg, pentobarbitone sodium 40 mg/kg indirect stimulation 10/min 5V 1 msec to anterior tibial muscle; at 1. AT_2 2 mg/kg close arterial. Rat 324 g, pentobarbitone sodium 40 mg/kg, i.p., indirect stimulation of sciatic gastrocnemius as per cat; at 1 AT_2 2 mg/kg close arterial.

AT_2. The effect on isolated frog rectus is a result of two actions. Small doses of AT_2 potentiate the contracture which is caused by ACh, carbachol, suxamethonium, and decamethonium, and inhibit the action of (+)−tubocurarine. Larger doses of AT_2

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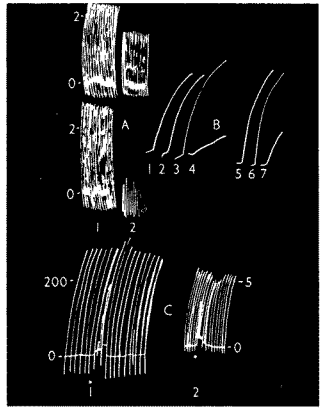

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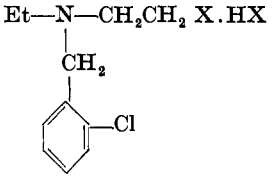
poison the muscle and antagonize the effect of ACh or K^+ (see Fig. 5B). The isolated rat diaphragm driven at 6 or 12 contractions per min is not directly affected by concentrations of up to 2×10^{-4} of AT_2. In the anaesthetized rat, close arterial injection of AT_2, 1–5 mg/kg, causes a short twitch response followed by a temporary diminution in force (see Fig. 5 C.2). The blocking action of gallamine triethiodide 3 mg/kg is diminished and that of suxamethonium 150 μg/kg increased. In the cat the effect of AT_2 1 mg/kg by close arterial injection is to produce a short burst of increased twitch activity in the indirectly stimulated anterior tibial muscle. The effect of suxamethonium is increased and that of gallamine diminished, again only to a slight degree.

This 2-halogenoalkylamine has a double action on muscle--a weak depolarizing effect on the synapse and a direct toxic action. The latter is an effect shared by the products of hydrolysis.

Discussion

The dependence of antiadrenaline activity on the position of the halogen substituent in the phenyl ring of 2-halogenoalkylamines of the structure



is amply confirmed. In all parent compounds examined [or the ethyleneiminium ions or E^+ (P_1–P_3)] the *p*-Cl structure is least active against adrenaline or noradrenaline. For 5-hydroxytryptamine, fewer cases have been examined. The relationship holds for bromoalkylamines; the iodoalkylamine has not been fully examined. The relative antiadrenaline activities of the *o*-Cl and *m*-Cl compounds vary. In the E^+, $o > m$; in the parent chloroalkylamines, $m > o$ against adrenaline and noradrenaline; $o > m$ in bromo- and iodoalkylamines against adrenaline but not noradrenaline. The differences are not great and *o* and *m* are clearly superior to *p*. A possible structure for the adrenaline receptor

has been discussed by Belleau,[2] who suggests that in any compounds with a substituent of sufficient size in the 4-position in the ring, activity will be reduced due to interference with the interaction with that part of the receptor which normally accepts the catechol nucleus of adrenaline. If the substituent in the benzyl moiety of the 2-halogenoalkylamine cannot rotate away from the subsidiary but important sites which receive the —OH groups of the catechol nucleus, the resulting repulsion from these parts will reduce anchoring of the antagonist molecule at this point, a necessary preliminary to alkylation of the site of attachment of the amine side-chain. This attractive theory is supported by the results for JFA_{8-16}. The receptor for 5-hydroxytryptamine must be similar to that for catechol amines in that the operative part for attachment is the phenolic moiety of the indole nucleus and not the whole indole nucleus since the catechol amines antagonize 5-hydroxytryptamine. The essential difference between 5-hydroxytryptamine and noradrenaline may be that the latter has two —OH groups and the former one.

The superior activity of Br- and I- to Cl-alkylamines which has been demonstrated before[16] is confirmed, but the relative importance of I and Br varies with the compound or the specific activity examined. The Br and I compounds release relatively large quantities of E^+ quickly whereas the Cl-compound releases E^+ slowly and in smaller amounts. A possible explanation of the reversal of the order of activity in the chloroalkylamine hydrochlorides ($m > o$; $JFA_{11} > JFA_{14}$) compared with the E^+ picrylsulphonates ($o > m$; $P_3 > P_2$) is that the net conversion to E^+ is less efficient with the *o*-chloro- than with the *m*-chloro-compound in the chloroalkylamine hydrochlorides. The rates of decomposition of E^+ in aqueous acetone are in the order $o > p > m$ and this may obtain *in vivo*. This general picture is confirmed in the series JFA_{28-36} but there are exceptions. In the *o*-tolyl series, the general rule for halogens that I > Br > Cl is not upheld and Cl > I against adrenaline and noradrenaline; again $o\text{-}CH_3 > p\text{-}CH_3$ in the tolyloxyethyl compounds where the halogen is Cl- and Br- but not I-. This rule holds in tests against 5-hydroxytryptamine but not histamine. The phenoxyethyl compounds are more active than the tolyloxyethyl compounds. A series of ratios of antiadrenaline activity for the *p*-, *m*- and *o*-Cl

compounds JFA_8, JFA_{11} and JFA_{14} in terms of the E^+ available from them in aqueous media at approximately blood temperature after a fixed time interval, compared with the activities of the derived E^+ picrylsulphonates, has been published.[3] The potency relation found is $P_3 > P_2 > P_1$, i.e. $o > m > p$ when injected as a salt of E^+. The relation of the parent compounds is $JFA_{11} > JFA_{14} > JFA_8$, i.e. $m > o > p$. It follows that while $o > m$ in terms of affinity, once the E^+ has reached the site of action, the *o*- form may be inferior to the *m*-form in terms of delivery of the E^+ to the site. *For drugs which form an unstable active intermediate* in vivo, *over-all affinity should be considered as the result of two processes—stability and access. Either may be crucial to final potency.*

The report[9] on the great activity of 9-fluorenamines is confirmed and extended. Increasing the size of the other substituent on the N-atom decreases activity (Et > benzyl) and a CH_3 group on the β carbon of the side chain also decreases activity against adrenaline and noradrenaline; the reverse holds against histamine and 5-hydroxytryptamine. The 9-fluorenamines are more potent than either 3-thionaphthenylmethylamines or, in particular, 2-thienylamines, for all four antagonisms. In all cases, the rule Br- or I- > Cl- obtains, with the exception of the 3-thionaphthenylmethylamines where Cl- > Br- or I- against adrenaline and noradrenaline.

Graham[3] has provided evidence that the active species from these compounds is E^+. It has been possible to select a compound (AT_{11}) which produces a solution which contains virtually no E^+, but piperazinium ion and some alcohol.[4] The inactivity of this solution is in agreement with the inactivity of dimers of certain derivatives of Dibenamine.[1]

Fingl and Gaddum[26] showed that, for Dibenamine, antagonistic potency is in the order A > NA > HT, and others[27] obtained the same result with J_{11}.[16] It can now be seen that this order is not universal for the group.

Local anaesthesia (intradermal) and general toxicity are probably dependent on different structure–action relations, e.g. in the *p*-Cl-benzyl series JFA_{8-10}, the potency runs Cl > I > Br for local anaesthesia and Br > Cl > I for lethality. Weighting one substituent on the nitrogen atom from ethyl to benzyl reduces

both activities. The nature of the other substituent does not have much effect on anaesthetic potency but does modify lethality, where 9-fluorenyl > thienyl. Insertion of oxygen as in phenoxyethyl- compounds increases anaesthetic potency and lowers toxicity, whereas methylation of the benzyl group reduces both activities. A p-CH_3 substituent is superior to o-CH_3 for anaesthesia but not for toxicity. These findings contrast with the antiadrenaline relationships.

Nickerson[28] has suggested certain tests to determine whether or not the antihistamine action of these compounds is 'competitive' in nature. The properties of AT_{11} agree with those indicative of a 'non-equilibrium' antagonist but there is no relationship between rate of onset of antagonism and duration of activity. The two-stage development of antagonism—firstly competitive as indicated for the related naphthylmethylhalogenoethylamines[16] and then 'non-equilibrium' as termed by Nickerson[28]—is evident. It has been shown[29] that increased K^+ increases the rate of recovery on washout of mepyramine but the present results show that, whereas the recovery after mepyramine with K^+ is permanent, after AT_{11} it is temporary. The effect of K^+ in temporarily overcoming the 'non-equilibrium' part of the activity of AT_{11} indicates that the site of this activity is probably on the cell membrane. The distinction between the type of antagonism to histamine displayed by 2-halogenoalkylamines and by mepyramine is only evident in a middle range of concentrations. The antagonism by AT_{11} and congeners is related to dose, up to the level of absolute block of the receptors, as it is with mepyramine, and is therefore not best described as 'non-equilibrium'. Its main feature is the long duration of action.

Certain central inhibitory actions of Dibenamine have been noted.[30] The results of a battery of tests indicate that compound AT_2 has central sedative actions.

Summary. A series of 2-halogenoalkylamines has been investigated for toxicity and antagonism to adrenaline, noradrenaline, histamine and 5-OH tryptamine. Selected compounds were tested for actions on the central nervous system, anticholinesterase activity, neuromuscular block and local anaesthetic properties. The importance of the position of substituents on the benzyl moiety of *N*-ethyl-*N*-benzyl-2-halogenoethylamines has been

demonstrated. The great potency of *N*-ethyl-*N*-9-fluorenamine-2-bromoethylamine HBr (AT_2) against adrenaline and that of *N*-ethyl-*N*-3-thionaphthylmethyl-2-bromoethylamine HBr (AT_{11}) against histamine has been revealed. The structure–action relations for several antagonisms are discussed. The mode of action against histamine is compared with that of mepyramine and tests designed to distinguish types of antagonism evaluated. The effect on the central nervous system of several 2-halogenoalkylamines is compared by a battery of tests and the sedative activity of AT_2 assessed.

Acknowledgements. Professor N. B. Chapman and colleagues of the Department of Chemistry, The University, Hull, provided all the compounds and the data on physical and kinetic properties, including Fig. 2. Dr. Richter of the Neuropsychiatric Research Unit, Whitchurch Hospital, Cardiff, gave facilities and assistance with the E.E.G. recording. Mr. Salway was responsible for many of the tests on spinal rats.

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